Listing of the Claims

The status of the claims is indicated below. No amendments to the claims are being made with this reply.

1. (Previously presented) A method of inducing and identifying a mutation in a gene subject to mutation in a eukaryotic cell, wherein the gene subject to mutation is operably linked to a promoter, and wherein the gene subject to mutation is within about two kilobases of the promoter, the method comprising

expressing a transgenic activation induced cytidine deaminase (AID) gene in the cell and expressing the gene subject to mutation in the cell,

establishing and culturing clonal colonies of the cell, and

identifying one or more clonal colonies that comprise a mutation in the gene subject to mutation.

2. (Previously presented) The method of claim 1, wherein the gene subject to mutation is also operably linked to an enhancer.

3. (Original) The method of claim 2, wherein the enhancer is an immunoglobulin enhancer.

4. (Previously presented) The method of claim 1, wherein the gene subject to mutation is between 10 bases and 2 kb in the 3' direction from the promoter.

5. (Previously presented) The method of claim 1, wherein the promoter is an immunoglobulin promoter.

6. (Previously presented) The method of claim 1, wherein a polyA mRNA of the gene subject to mutation is synthesized in the cell, the polyA mRNA of the gene comprising at least 0.01% of total polyA mRNA in the cell.

7. (Previously presented) The method of claim 6, wherein the polyA mRNA of the gene subject to mutation comprises at least 0.1% of total polyA mRNA in the cell.

8. (Previously presented) The method of claim 6, wherein the polyA mRNA of the gene subject to mutation comprises at least 0.5% of total polyA mRNA in the cell.

9. (Previously presented) The method of claim 6, wherein the polyA mRNA of the gene subject to mutation comprises at least 1% of total polyA mRNA in the cell.

10-12. (Canceled)

13. (Previously presented) The method claim 1, wherein the AID gene is flanked at the 5' end by a sequence foreign to the cell, wherein the sequence foreign to the cell is at least 200 bp long.

14. (Canceled)

15. (Original) The method of claim 13, wherein the sequence foreign to the cell is at least 2000 bp long.

16-17. (Canceled)

18. (Previously presented) The method of any one of claims 1, 13, or 15, wherein the cell is a yeast cell.

19. (Previously presented) The method of any one of claims 1, 13, or 15, wherein the cell is a vertebrate cell.

20. (Original) The method of claim 19, wherein the cell is a mammalian cell.

21. (Original) The method of claim 20, wherein the cell is a B cell.

22. (Original) The method of claim 20, wherein the cell is a hybridoma.

23. (Previously presented) The method of claim 1, wherein the cell is a human cell.

24. (Previously presented) The method of claim 1, wherein the gene is an antibody gene.

25. (Previously presented) The method of claim 1, wherein the gene encodes a protein selected from the group consisting of an enzyme, a transcription factor, a cytokine, and a structural protein.

26-57. (Canceled)

58. (Previously presented) A method of inducing and identifying a mutation in an antibody gene in a eukaryotic cell, the method comprising

expressing a transgenic AID gene in the cell and expressing the antibody gene in the cell,

establishing and culturing clonal colonies of the cell, and

identifying one or more clonal colonies that comprise a mutation in the antibody gene.

59-96. (Canceled)

97. (Previously presented) A method of inducing and identifying a class switch in an antibody heavy chain gene in a eukaryotic cell, wherein the cell is a myeloma, the method comprising

expressing a transgenic AID gene in the cell and expressing the antibody heavy chain gene in the cell,

establishing and culturing clonal colonies of the cell, and

identifying one or more clonal colonies comprising the class switch in the antibody heavy chain gene.

98-124. (Canceled)

125. (Previously presented) A method of altering an affinity or a specificity of a monoclonal antibody to a first antigen, or altering a cross reactivity of the monoclonal antibody to a second antigen, wherein the monoclonal antibody is produced by a eukaryotic cell, and wherein the cell is capable of expressing a transgenic AID gene under inducible control, the method comprising

(a) expressing the AID gene in the eukaryotic cell for a time and under conditions sufficient to induce a mutation in a gene encoding the monoclonal antibody;

(b) suppressing expression of AID gene in the eukaryotic cell;

(c) establishing clonal colonies of the cell; and

(d) determining whether the monoclonal antibody produced by any of the clonal colonies of the cell has altered affinity or specificity to the first antigen, or altered cross reactivity to the second antigen.

126-261. (Canceled)

262. (Previously presented) The method of claim 1, wherein the gene subject to mutation is integrated into the genome of the cell.

263. (Previously presented) The method of claim 1, wherein the gene subject to mutation is present extrachromosomally in the cell.

264. (Previously presented) The method of claim 1, wherein the gene subject to mutation is a native gene.

265. (Previously presented) The method of claim 1, wherein the gene subject to mutation is a transgene.

266. (Previously presented) The method of claim 1, wherein expression of the AID gene is constitutive.

267. (Previously presented) The method of claim 1, wherein expression of the AID gene is inducible.

268. (Previously presented) The method of claim 267, wherein the inducible AID expression is under control of a tet system or ecdysone receptor system.

269. (Previously presented) The method of claim 58, wherein the antibody gene encodes at least a portion of an antibody that binds to an antigen.

270. (Previously presented) The method of claim 58, wherein expression of the AID gene is constitutive.

271. (Previously presented) The method of claim 58, wherein expression of the AID gene is inducible.

272. (Previously presented) The method of claim 271, wherein the inducible AID expression is under control of a tet system or ecdysone receptor system.

273. (Previously presented) The method of claim 58, wherein the antibody gene is a single chain antibody.

274. (Previously presented) The method of claim 58, wherein the antibody gene is a multivalent antibody.

275. (Previously presented) The method of claim 58, wherein the antibody gene is a catalytic antibody.

276. (Previously presented) The method of claim 58, wherein the antibody gene is selected from the group consisting of a human or humanized antibody, a mouse antibody, a rabbit antibody, and a hamster antibody.

277. (Previously presented) The method of claim 269, wherein the mutated antibody gene encodes at least a portion of an antibody that has a higher affinity for the antigen than the antibody before the mutation.

278. (Previously presented) The method of claim 269, wherein the mutated antibody gene encodes at least a portion of an antibody that has a lower affinity for the antigen than the antibody before the mutation.

279. (Previously presented) The method of claim 269, wherein the mutated antibody gene encodes at least a portion of an antibody that has a higher specificity for the antigen than the antibody before the mutation.

280. (Previously presented) The method of claim 269, wherein the mutated antibody gene encodes at least a portion of an antibody that has a lower specificity for the antigen than the antibody before the mutation.

281. (Previously presented) The method of claim 269, wherein the mutated antibody gene encodes at least a portion of an antibody that has altered cross-reactivity for a second antigen than the antibody before the mutation.

282. (Previously presented) The method of claim 281, wherein the mutated antibody gene encodes at least a portion of an antibody that has increased cross reactivity for the second antigen than the antibody before the mutation.

283. (Previously presented) The method of claim 281, wherein the mutated antibody gene encodes at least a portion of an antibody that has decreased cross reactivity for the second antigen than the antibody before the mutation.

284. (Previously presented) The method of claim 58, wherein both a heavy chain and a light chain of the antibody gene are mutated.

285. (Previously presented) The method of claim 58, wherein the cell is a yeast cell.

286. (Previously presented) The method of claim 58, wherein the cell is an insect cell.

287. (Previously presented) The method of claim 58, wherein the cell is a vertebrate cell.

288. (Previously presented) The method of claim 287, wherein the cell is a mammalian cell.

289. (Previously presented) The method of claim 125, wherein steps (a) through (d) are repeated with a clonal colony that has altered affinity or specificity to the antigen, or altered cross-reactivity to the second antigen.

290. (Previously presented) The method of claim 125, wherein the clonal cells are enriched for cells producing high affinity antibodies by FACS.

291. (Previously presented) The method of claim 125, wherein the inducible AID gene expression is under control of a tet system or ecdysone receptor system.

292. (Previously presented) The method of claim 125, wherein the AID gene is flanked by a sequence foreign to the cell, wherein the sequence foreign to the cell is at least 200 bp long.

293. (Previously presented) The method of claim 125, wherein the monoclonal antibody is selected from the group consisting of a human or humanized antibody, a mouse antibody, a rabbit antibody, and a hamster antibody.

294. (Previously presented) The method of claim 125, wherein the mutated monoclonal antibody gene encodes at least a portion of an antibody that has higher affinity for the first antigen than the antibody before the mutation.

295. (Previously presented) The method of claim 125, wherein the mutated monoclonal antibody gene encodes at least a portion of an antibody that has lower affinity for the first antigen than the antibody before the mutation.

296. (Previously presented): The method of claim 125, wherein the mutated monoclonal antibody gene encodes at least a portion of an antibody that has higher specificity for the first antigen than the antibody before the mutation.

297. (Previously presented): The method of claim 125, wherein the mutated monoclonal antibody gene encodes at least a portion of an antibody that has lower specificity for the first antigen than the antibody before the mutation.

298. (Previously presented): The method of claim 125, wherein the mutated monoclonal antibody gene encodes at least a portion of an antibody that has altered cross-reactivity for the second antigen than the antibody before the mutation.

299. (Previously presented): The method of claim 298, wherein the mutated monoclonal antibody gene encodes at least a portion of an antibody that has increased cross-reactivity for the second antigen than the antibody before the mutation.

300. (Previously presented). The method of claim 298, wherein the mutated monoclonal antibody gene encodes at least a portion of an antibody that has decreased cross-reactivity for the second antigen than the antibody before the mutation.

301. (Previously presented) The method of claim 1, wherein the clonal colonies that comprise a mutation in the gene subject to mutation are separated from the rest of the cells and propagated to produce mutant protein.

302. (Previously presented) The method of claim 58, wherein the clonal colonies that comprise a mutation in the antibody gene are separated from the rest of the cells and propagated to produce mutant antibody.

303. (Previously presented) The method of claim 125, wherein the clonal colonies that comprise a mutation in the monoclonal antibody gene are separated from the rest of the cells and propagated to produce mutant monoclonal antibody.

304. (Previously presented) The method of claim 1, wherein the cell is a non-B cell.

305. (Previously presented) The method of claim 58, wherein the cell is a non-B cell.

306. (Previously presented) The method of claim 125, wherein the cell is a non-B cell.

307. (Previously presented) The method of claim 97, wherein the cell is a hybridoma.